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## RADIOBIOLOGY SPECIAL FEATURE: REVIEW ARTICLE

# An arranged marriage for precision medicine: hypoxia and genomic assays in localized prostate cancer radiotherapy

<sup>1,2,3</sup>RG BRISTOW, MD, PhD, FRCPC, <sup>1,2,3</sup>A BERLIN, MD and <sup>1,2,3</sup>A DAL PRA, MD

<sup>1</sup>Princess Margaret Cancer Center (University Health Network), Toronto, ON, Canada

<sup>2</sup>Department of Radiation Oncology, University of Toronto, Toronto, ON, Canada

<sup>3</sup>Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

Address correspondence to: Professor Robert G. Bristow

E-mail: [rob.bristow@rmp.uhn.on.ca](mailto:rob.bristow@rmp.uhn.on.ca)

### ABSTRACT

Prostate cancer (CaP) is the most commonly diagnosed malignancy in males in the Western world with one in six males diagnosed in their lifetime. Current clinical prognostication groupings use pathologic Gleason score, pre-treatment prostatic-specific antigen and Union for International Cancer Control-TNM staging to place patients with localized CaP into low-, intermediate- and high-risk categories. These categories represent an increasing risk of biochemical failure and CaP-specific mortality rates, they also reflect the need for increasing treatment intensity and justification for increased side effects. In this article, we point out that 30–50% of patients will still fail image-guided radiotherapy or surgery despite the judicious use of clinical risk categories owing to interpatient heterogeneity in treatment response. To improve treatment individualization, better predictors of prognosis and radiotherapy treatment response are needed to triage patients to bespoke and intensified CaP treatment protocols. These should include the use of pre-treatment genomic tests based on DNA or RNA indices and/or assays that reflect cancer metabolism, such as hypoxia assays, to define patient-specific CaP progression and aggression. More importantly, it is argued that these novel prognostic assays could be even more useful if combined together to drive forward precision cancer medicine for localized CaP.

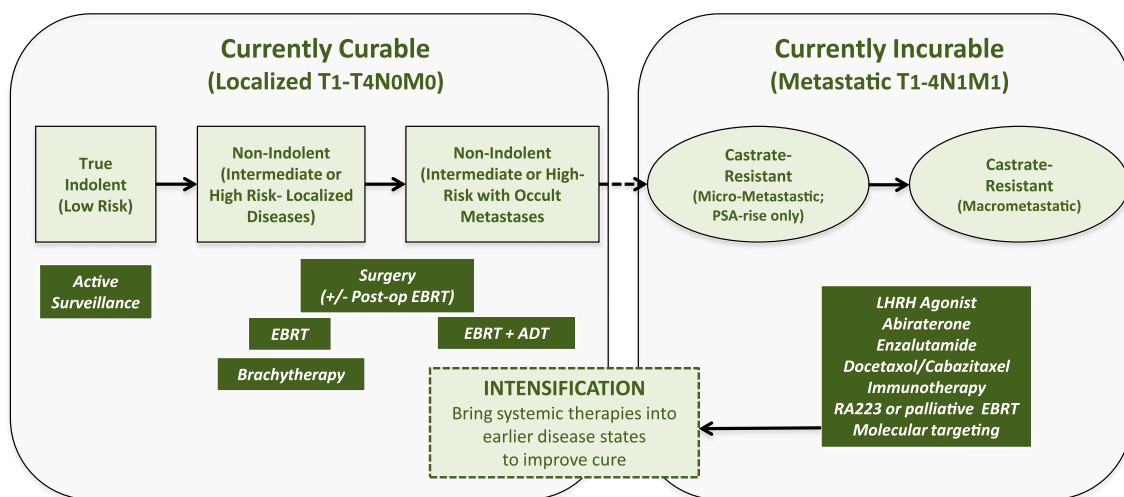
### BACKGROUND: THE NEED FOR NOVEL BIOLOGICAL END POINTS FOR PROSTATE CANCER PROGNOSIS

Prostate cancer (CaP) is the most commonly diagnosed malignancy in males in the Western world, as >500 000 cases are diagnosed annually and 1 in 34 will die of metastatic disease.<sup>1</sup> Treatment options for localized CaP depend on the Union for International Cancer Control-TNM staging [*i.e.* extent of tumour (local), nodal and distant metastatic cancer burden] of the disease. Using the clinical prognostic variables, local T-category, pre-treatment serum prostate-specific antigen (PSA) and pathologic Gleason score (GS; usually ranging from 5–10) males with localized CaP (*e.g.* T1–T4N0M0) are placed in low-, intermediate- and high-risk prognostic groups.<sup>2,3</sup> These risk groups predict for biochemical failure based on a post-treatment rise in PSA (also referred to as biochemical relapse-free rate) and CaP-specific mortality (PCSM) after local therapies with curative intent.<sup>2–4</sup>

Active surveillance (AS) is a treatment option for low-risk and probably indolent CaPs, which have PSA values <10 ng ml<sup>-1</sup> associated with small volume of GS6 or less

in patients' diagnostic biopsies.<sup>5,6</sup> Radical prostatectomy or radiotherapy [RT; using either external beam RT (EBRT) or brachytherapy] constitutes the major treatment options for non-indolent intermediate-risk CaP (*e.g.* T1–T2 lesions, PSA <20 ng ml<sup>-1</sup> and GSs 6 or 7; [Figure 1](#)). The final choice of treatment will depend on patient preference and other considerations (*e.g.* operative risk, comorbidities, obstructive urinary symptoms, contraindications to RT *etc.*).<sup>1</sup> However, patients with high-risk or locally advanced disease (*e.g.* T3–T4 lesions outside the prostate gland and/or GSs ≥8 and/or PSA values >20 ng ml<sup>-1</sup>) undergo combined modality treatment consisting of either adjuvant or salvage RT following surgery to offset local failure, or undergo combined use of EBRT with androgen deprivation therapy (ADT) to offset the risk of subclinical metastases.<sup>1,7,8</sup> In males who develop castrate-resistant and metastatic disease ([Figure 1](#)), palliative options include systemic treatment using ADT (luteinizing hormone-releasing hormone agonists/antagonists with secondary hormonal manipulation using enzalutamide or abiraterone), chemotherapy (using docetaxel or cabazitaxel), systemic radionuclides (Radium-223), immunotherapy (Sipuleucel-T) and/or targeted palliative RT (*e.g.* 8 Gy single dose or 20–30 Gy in daily fractions).<sup>9,10</sup>

Figure 1. Curative and non-curative states in prostate cancer. Localized prostate cancers (CaPs) can be divided into low-, intermediate- and high-risk (including locally advanced) groups using T-category, pre-treatment prostate-specific antigen (PSA) level and the pathologic Gleason score. These groups have increasing probability of CaP-specific mortality. Low-risk tumours can be aggressively followed using active surveillance. By contrast, intermediate-risk tumours are treated with surgery, external beam radiotherapy (EBRT) or brachytherapy. In cases where a local recurrence occurs after surgery, patients can be treated with post-operative EBRT and convert a local failure into a cure. In high-risk CaP, there is an increased probability for occult systemic metastases, therefore systemic androgen deprivation [androgen-deprivation therapy (ADT)] is used in combination with EBRT. Palliative systemic therapy is the mainstay for patients with castrate-resistant disease in the micrometastatic or macrometastatic stages to increase progression-free survival by months. These therapies include additional ADT (including the use of newer agents, such as abiraterone and enzalutamide), chemotherapy, immunotherapy, systemic radionucleotides (RA223) and use of bespoke molecular-targeted agents. It is argued that an understanding of the genomic and microenvironmental factors that lead to occult metastases could drive intensification protocols using systemic agents in the localized CaP setting to improve the cure rates with radiotherapy and surgery. LHRH, luteinizing hormone-releasing hormone; Post-op, post-operative; RA223, radium-223.



Despite a multitude of treatment options, there are no individualized clinical tests that absolutely tell which patients are *unlikely* to fail local treatment from those patients who are *most likely* to fail local treatment within a given clinical risk category. This problem is illustrated by the fact that despite the use of stringent clinical criteria to place patients into clinical prognostic groups, 30–50% of males can still fail precision RT or surgery owing to local resistance and/or systemic spread.<sup>1–3</sup> Despite the publication of Phase III dose-escalated EBRT clinical trials in CaP designed to counteract failure due to CaP radioresistance, none of these trials have shown benefit in decreasing PCSM.<sup>8</sup> The lack of an effect on survival with EBRT dose escalation can be explained by the fact that in a significant proportion of patients, treatment failure is due to the presence of occult systemic disease rather than local resistance, and that these patients need to be treated with intensification of systemic therapy not EBRT dose intensification, to decrease CaP mortality.<sup>1,8</sup> Personalized CaP medicine therefore requires genomic- or biology-based biomarkers, in addition to existing clinical biomarkers, to explain interpatient heterogeneity in outcomes. Furthermore, even if an increased probability of occult metastases can be predicted, even more biomarkers will be required to favour the use of one systemic agent *vs* another, let alone the scheduling of these agents relative to each other (Figure 1).<sup>10</sup>

An additional complication to personalized medicine is the knowledge that many low-risk CaPs are indolent and that their overtreatment results in significant morbidity.<sup>4,5,11</sup> For example,

two-thirds of low-risk CaPs have an indolent course that can be followed without radical treatment when appropriately placed into AS protocols, thereby preventing the side effects and costs of RT or surgery. The corollary is that one-third of these low-risk patients are being inaccurately classified as having indolent cancers and require treatment.<sup>5</sup> On an individual basis, there are no assays that can predict with confidence the need for therapy in low-risk CaP.

So, how do we move forward in precision medicine for CaP using precision RT when faced with such clinical conundrums? One approach is to take advantage of technological advances in genomic medicine to determine patient-specific CaP genomics that reflect tumour progression and metastatic disease in addition to novel biology.<sup>12,13</sup> State-of-the-art whole-genome sequencing technologies have the capacity for generating a breathtaking amount of genomic data (in excess of 10 billion bases per day) at a fraction of the cost than a decade ago. DNA- and RNA-based prognostic tests to predict CaP recurrence are being actively developed within the industry and academia for clinical use. Finally, there is also a rich history in radiation oncology for characterizing the tumour microenvironment, including assays for subregions of hypoxia within localized CaPs, which have a prognostic impact. This article will now discuss the potential of genomic and hypoxia assays to help attain the goal of implementing precision cancer medicine for patients undergoing curative RT for CaP.

## TUMOUR HYPOXIA MEASUREMENTS ARE PROGNOSTIC IN LOCALIZED PROSTATE CANCER

The tumour microenvironment is characterized by subregions of nutrient deprivation, low extracellular pH, high interstitial fluid pressure and hypoxia. Hypoxic areas arise in tumours when oxygen consumption rate exceeds that of supply. The blood vessels within a tumour microenvironment are usually irregularly organized and have abnormal architecture such that tumours will contain regions where the partial oxygen concentration ( $pO_2$ ) is significantly  $<5$  mmHg (*i.e.* normal tissues range from 10 to 80 mmHg).<sup>14</sup> Tumour cells that lie beyond the diffusion distance for oxygen ( $>100\ \mu\text{m}$  away from blood vessels) can quickly outstrip blood supply and are exposed to chronically low oxygen tensions for hours to days; this is often referred to as “chronic hypoxia”.<sup>14,15</sup> Tumour cells remain hypoxic until they die (due to lack of oxygen or nutrients) or are reoxygenated. Hypoxia can also be transiently “acute” or “cycling” due to acute perfusion changes in the tumour vasculature.<sup>14,15</sup> Tumours therefore contain a mixture of acute (cycling) and chronic hypoxia subregions with varying biology and varying effects on tumour cell radiosensitivity and genomic stability.<sup>14</sup>

Intratumoural hypoxia limits the effectiveness of RT and chemotherapy. Cells that are hypoxic or anoxic are usually two to three times more resistant to ionizing radiation when compared with oxic cells unless they are DNA repair deficient (see hypoxia section below).<sup>14</sup> Chemotherapy-related tumour cell kill in this scenario is also limited owing to poor drug distribution and decreased tumour cell proliferation that limits the effectiveness of S-phase-specific chemotherapeutics.<sup>14</sup> Therapy-resistant cells can adapt to hypoxic regions and result in cycles of selection for aggressive mutator phenotypes with faulty DNA repair and increased genetic instability.<sup>15,16</sup> Pre-clinical data have also linked tumour cell hypoxia to increased experimental and spontaneous metastasis.<sup>14</sup> Metastasis is a multistep process that involves intravasation through the basement membrane and extracellular matrix into the host vasculature, extravasation through vessel walls and then forming a new nidus with the organ of metastatic spread, and then tumour angiogenesis during secondary tumour growth. Indeed, hypoxia alters metastasis gene expression including that of E-cadherin (cell–cell contact), urokinase-type plasminogen activator receptor (degradation of extracellular matrix proteins), hepatocyte growth factor (cellular motility) and vascular endothelial growth factor (VEGF; angiogenesis and vascular permeability).<sup>14</sup> Therefore, pre-clinical data support two general aspects relating to the resistance of hypoxic tumours during RT: increased local tumour cell radioresistance<sup>1</sup> and/or an increased capacity for systemic metastases.<sup>2</sup>

Clinical studies that have attempted to directly measure the level of hypoxia in CaP have used  $pO_2$  electrodes, hypoxia imaging [positron emission tomography (PET)] and immunohistochemistry (IHC)<sup>17–25</sup> (Table 1). These studies support the concept that hypoxic subregions exist within localized CaPs and are associated with higher rates of biochemical failure following surgery or RT. For example, a prospective clinical trial showed that localized CaPs have uptake of the hypoxia biomarker, pimonidazole, and that this uptake correlated with GS but not vascularity.<sup>18</sup> Turaka et al<sup>23</sup> showed that decreased prostate-to-muscle oxygen ratio was an important

predictor of early biochemical recurrence following brachytherapy secondary to occult metastases at the time of treatment. In the largest clinical study of CaP hypoxia using direct  $pO_2$  measurements, Milosevic et al<sup>21</sup> showed that hypoxia is associated with both early biochemical relapse and local recurrence in the prostate gland.

Hypoxia leads to an upregulation of the transcription factor, hypoxia inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ), which in turn can increase the expression of downstream proteins such as VEGF, carbonic anhydrase IX (CAIX), glucose transporter 1 (GLUT-1) and osteopontin (OPN).<sup>22,26</sup> Vergis et al<sup>24</sup> used an IHC-based approach to show that HIF-1 $\alpha$ , VEGF and OPN (for surgical patients) and HIF-1 $\alpha$  and VEGF (for RT patients) predicted biochemical failure independent of the clinical tumour stage, GS, serum PSA and RT dose. However, a study from the University of Michigan (Ann Arbor, MI) using PET-fluoroazomycin arabinoside analogue (FAZA; as a hypoxia marker) failed to show FAZA uptake or CAIX staining in CaP, suggesting that these biomarkers have little utility in prognostication.<sup>19</sup> When taken together, the observation of a low  $pO_2$  predicting early failures in the first 2 years following either RT or surgical treatment suggests that hypoxia is associated with a metastatic phenotype.<sup>21,23,24</sup> Such hypoxic tumours will require treatment intensification (discussed below) when using EBRT to offset both local radioresistance and systemic metastases.

Despite these data, hypoxia-targeted therapy is still not a standard current cancer treatment. Agents that may be useful in this context might include the use of ADT (given the ability of androgen suppression to improve CaP oxygenation), molecular-targeted agents or hypoxia-targeted systemic agents.<sup>15</sup> Importantly, as we will see below, hypoxia can also drive genetic instability by inhibiting DNA repair.<sup>27</sup> Understanding these additional consequences of the hypoxic microenvironment on the development of genetic instability may give novel treatment approaches to combat hypoxia-associated resistance.

## GENETIC INSTABILITY AND GENOMIC ASSAYS IN LOCALIZED PROSTATE CANCER

Chromosomal instability and aneuploidy are associated with cancer progression and adverse prognosis in CaP.<sup>28–32</sup> Patients with tetraploid or aneuploid CaP tumours have increased mortality following radical prostatectomy when compared with patients whose CaP tumours are diploid.<sup>30</sup> The specific genomic events that might link to this aspect of aggression are now being understood in the context of abnormal gene copy number loss, gene mutation and abnormal gene expression relating to oncogenes and tumour suppressor genes. Hypothesis-based studies have used array comparative genomic hybridization (using DNA from diagnostic CaP biopsies prior to therapy) to associate specific gene copy number alterations with prognosis following EBRT or radical prostatectomy. Copy number loss of the tumour suppressor genes novel human prostate-specific, androgen-related homeobox gene (*NKX3.1*) or Phosphatase and tensin homologue (*PTEN*) or the androgen synthesis genes steroidogenic acute regulatory protein (*StAR*) and hydroxysteroid (17- $\beta$ ) dehydrogenase 2 (*HSD17B2*) are novel and independent genomic prognostic factors (hazard ratio ranges from 2 to 4 for failing local therapy). When associated with copy number gain of the proto-oncogene *cMYC*,<sup>33–35</sup> *NKX3.1* loss was also associated with local radioresistance. Furthermore, males who

Table 1. Clinical studies of hypoxia assays and prognosis in localized prostate cancer

Study	N	T-category	Assay	Prognostic value and details
Turaka <i>et al</i> <sup>23</sup>	57	cT1–3	pO <sub>2</sub> probe	Prognostic: lower prostate/muscle pO <sub>2</sub> ratio predicted early biochemical failure after brachytherapy
Milosevic <i>et al</i> <sup>21</sup>	247	cT1–2	pO <sub>2</sub> probe	Prognostic: largest study showing that hypoxia predicted early biochemical relapse after radiotherapy and local recurrence
Vergis <i>et al</i> <sup>24</sup>	201 (RT); 289 (surgery)	cT1–3	IHC-VEGF, HIF-1 $\alpha$ , OPN	Prognostic: increased expression of VEGF, HIF-1 $\alpha$ and, for patients treated with surgery, OPN identified patients at high risk of biochemical failure
Carnell <i>et al</i> <sup>18</sup>	43	cT1–3	IHC-PIMO	Not tested, but a positive correlation of PIMO +3 binding with Gleason score was demonstrated
Boddy <i>et al</i> <sup>17</sup>	149	cT1–3	IHC-VEGF, HIF-1 $\alpha$	Not prognostic: there was a significant correlation between HIF-1 $\alpha$ and HIF-2 $\alpha$ expression, and with AR and VEGF expression. VEGF was also significantly related to the androgen receptor, whereas PHD2 was inversely related to HIF-2 $\alpha$ expression. No significant association was shown between HIF-1 $\alpha$ or HIF-2 $\alpha$ and time to PSA recurrence
Green <i>et al</i> <sup>20</sup>	50	cT3	IHC	Prognostic: high VEGF expression was associated with lower disease-specific survival
Thoms <i>et al</i> <sup>22</sup>	199 (T1–3); 37 (M1)	cT1–T3	ELISA-OPN	Not prognostic: within localized prostate cancers plasma OPN was not predictive of more aggressive disease or response to radiotherapy or hormone therapy
Weber <i>et al</i> <sup>25</sup>	103	cT1–3	IHC	Prognostic: high nuclear expression of HIF-1 $\alpha$ and low EGFR expression was associated with a good prognosis in patients treated with RT $\pm$ ADT
Garcia-Parra <i>et al</i> <sup>19</sup>	14	pT2b–T3a	PET-FAZA + IHC	Not prognostic: negative <sup>18</sup> F-FAZA accumulation and CAIX staining in primary prostate cancer despite documented large lesions (up to 4 cm). HIF-1 staining was positive and independent of Gleason score

ADT, androgen-deprivation therapy; AR, androgen receptor; CAIX, carbonic anhydrase IX; cT, clinical T-category; EGFR, epidermal growth factor receptor; ELISA, enzyme-linked immunosorbent assay; <sup>18</sup>F-FAZA, <sup>18</sup>F-labelled fluoroazomycin arabinoside; HIF-1, hypoxia-induced factor 1; IHC, immunohistochemistry; OPN, osteopontin; PET, positron emission tomography; PHD, prolyl hydroxylase enzyme; PIMO, pimonidazole; pO<sub>2</sub>, partial oxygen concentration; PSA, prostate-specific antigen; pT, pathologic T-category; RT, radiotherapy; VEGF, vascular endothelial growth factor. pO<sub>2</sub>, measured with pO<sub>2</sub> electrode.

carry germ-line mutations in the breast cancer 2, early onset (*BRCA2*) tumour suppressor gene develop aggressive CaPs (higher GSs and increased nodal metastases) that have very poor prognosis following surgery or RT.<sup>36,37</sup> Surprisingly, the presence or absence of a fusion gene between transmembrane protease serine 2 and the ETS-related gene (*TMPRSS2-ERG*) fusion (found in >50% of all CaP patients) is not prognostic.<sup>38,39</sup>

Unbiased genome-wide signatures based on DNA or RNA indices have recently been developed to predict PSA recurrence in the post-operative setting. A set of DNA-based biomarkers (genomic evaluators of metastatic CaP) has improved utility

over the sole use of clinical recurrence nomograms (*e.g.* Kattan nomogram) in the prediction of recurrence following surgery.<sup>40</sup> Recent evidence also suggests that miRNAs may also have prognostic value, although only a limited number of studies have correlated genome-wide analysis of miRNA species with differential prognosis.<sup>41–43</sup> High levels of miR-96 were prognostic of biochemical recurrence in a series of 155 radical prostatectomies.<sup>41</sup> Similarly, others have noted an independent prognostic value (*e.g.* independent from currently utilized clinical parameters of PSA, T-category and GS) for miR-191, miR-145, miR-100 and miR-122, many of which are arranged in genomic clusters.<sup>42,43</sup>

Table 2. DNA- and RNA-based prognostic signatures for localized prostate cancer

Signature (DNA or RNA)	Signature development cohort	Outcomes predicted	Validation in separate cohorts (yes/no)	Evaluated in other treatment modality cohorts	References
DNA-based CNAs for NKX3.1, PTEN, cMYC, StAR	Pre-radiotherapy	BCR	No	Yes: surgery	Zafarana et al <sup>35</sup> ; Locke et al <sup>33,34</sup>
DNA-based CNA clusters (6 clusters)	Post-surgery recurrence	BCR	No	No	Taylor et al <sup>44</sup>
RNA 22-gene expression signature	Post-surgery recurrence	M, PCSS, OS	Yes (two cohorts)	No	Cooperberg et al <sup>45</sup> ; Erho et al <sup>46</sup>
RNA 31-gene expression signature	Post-surgery recurrence post-TURP recurrence	BCR, PCSS	Yes (three cohorts)	Yes (conservatively managed and radiotherapy)	Cuzick et al <sup>47</sup>
RNA 32-gene expression signature	Post-surgery recurrence	BCR, M	Yes (one cohort)	No	Wu et al <sup>48</sup>

BCR, biochemical recurrence; cMYC, proto-oncogene cMYC; CNA, gene copy number; M, metastases; NKX3.1, novel human prostate-specific, androgen-regulated homeobox gene; OS, overall survival; PCSS, prostate specific-cancer survival; PTEN, phosphatase and tensin homolog; TURP, transurethral resection of the prostate.

Major research efforts have led to the development of RNA-based signatures to better stratify patients between indolent vs aggressive CaPs (Table 2). A 31-gene signature of biochemical recurrence following radical prostatectomy has been reported based on RNA expression of cell cycle progression genes;<sup>47</sup> this prognostic assay was also validated in the setting of recurrence following EBRT.<sup>49,50</sup> A 22-gene RNA expression panel has been similarly validated across multiple independent cohorts by several groups.<sup>45,46,51</sup> Likewise, Wu et al<sup>48</sup> have reported a 32-gene RNA expression signature that is prognostic of biochemical recurrence and metastatic disease following radical prostatectomy. Using DNA-based indices, Taylor et al<sup>44</sup> have shown independent prognostic utility for six genetic clusters that are prognostic for biochemical recurrence, independent of the GS.

Additional DNA epigenetic modifications may drive individualized CaP biology and progression. Using high-throughput genome sequencing and DNA methylation analyses, >147 000 cancer-associated epigenetic alterations were observed in 51 tumour and 53 benign prostate samples; the specific nature of these alterations were dependent on the presence or absence of a TMPRSS2-ERG rearrangement.<sup>52</sup> This observation of differential methylation events in fusion-negative tumours (based on enhancer of zeste homologue 2 (*EZH2*) gene activation) explains CaP carcinogenesis in the 50% of patients who are TMPRSS2-ERG fusion-negative. Systematic overviews of studies for the prognostic role of specific gene methylation (e.g. *GSTP1*, *APC*, *RAR-β*, *RASSF1A*, *PITX2*, *CCND2*, *EDNRB* and *HOX* family of genes) in CaP concluded that their prognostic roles are still unknown and require further validation in large clinical cohorts.<sup>53,54</sup>

Integrating genome-, epigenome-, transcriptome- and proteome-wide data sets to iterate a multimodal genetic test will no doubt improve subgroup prognostication.<sup>55,56</sup> Therefore, similar to hypoxia assays, patient-specific indices of genetic instability may

be utilized to further define aggressive subsets of CaP for treatment intensification<sup>27</sup> (Figure 2). But is there a biological link between genetic instability and hypoxia that leads to adverse prognosis? We will now discuss the potential interplay between hypoxia, DNA repair and genetic instability.

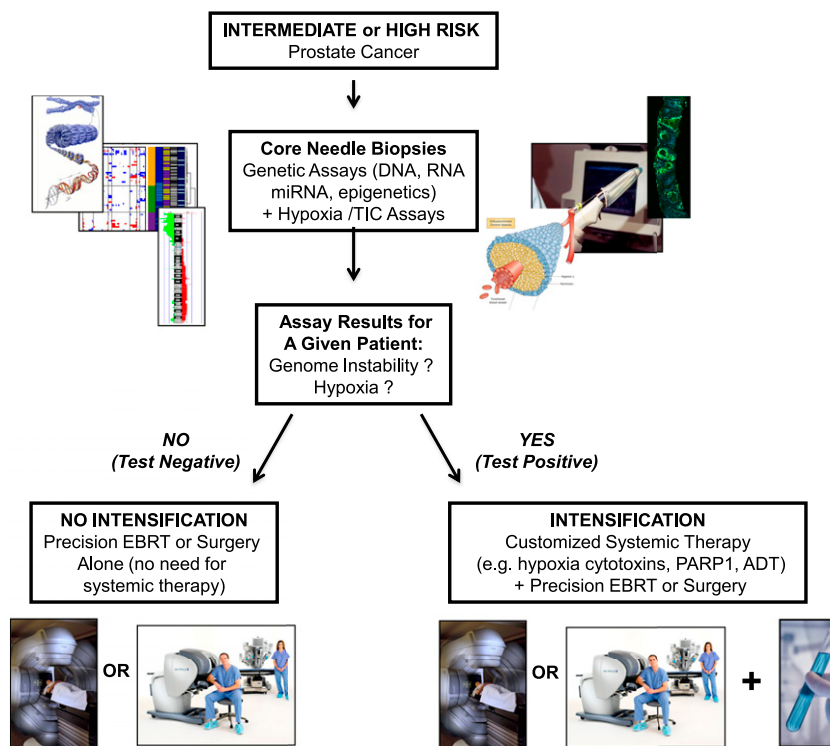
### HYPOXIA AND GENOMIC INSTABILITY IN PROSTATE CANCER: A NOVEL THERAPEUTIC TARGET?

One model of interaction between the CaP tumour micro-environment and CaP genomics is that hypoxic tumour cells have down-regulated DNA repair function (e.g. decreased capacity for the repair of DNA double strand breaks; DSBs) in addition to any genetic instability due to oncogene activation or tumour suppressor gene inactivation.<sup>14,27</sup> Such repair-deficient hypoxic tumour cells could adapt to low oxygen levels and acquire an aggressive “mutator” phenotype leading to treatment resistance and metastases.<sup>27</sup> For example, there are two major pathways of DSB repair: non-homologous end-joining (NHEJ; active throughout the cell cycle) and homologous recombination (HR; requiring a homologous chromosome available only during S and G2 phases of the cell cycle).<sup>57</sup> Hypoxia causes decreased transcription and translation of a series of HR and NHEJ genes, including *Rad51*, *BRCA1*, *BRCA2* and DNA-protein kinase catalytic subunit (*DNA-PKcs*). Furthermore, functional studies using isogenic cells have reported hypoxia-induced NHEJ and HR defects.<sup>15,16,58–60</sup> As a consequence, despite lower levels of initial DSB formation following ionizing radiation (IR) in hypoxic tumour cells, hypoxia-induced defects in DSBs repair increased the level of unrepaired DSBs and chromosomal aberrations at the first mitosis post-irradiation.<sup>27,59</sup> If even a fraction of these hypoxic mutant tumour cells survive subsequent cell division, unstable genetic mutants could undergo clonal selection.

Hypoxia induces activation of common fragile sites throughout the genome (i.e. chromosomal regions prone to breakage) and



Figure 2. Combining genomics and hypoxia assays to drive personalized prostate cancer medicine. Genomic signatures (DNA, RNA, epigenetic or miRNA-based) could be combined with hypoxia assays (using imaging such as positron emission tomography-fluoroazomycin arabinoside or intrinsic/extrinsic markers *in situ*) to triage patients with low probability of systemic metastases to local treatment alone and patients with high probability of metastases to local treatment plus systemic treatment (e.g. combined modality therapy). Systemic treatments could include those shown in Figure 1 that are currently used for metastatic disease, hypoxia-specific cytotoxins or novel agents designed to target abnormal signalling or DNA repair pathways based on susceptibility biomarkers. ADT, androgen-deprivation therapy; EBRT, external beam radiotherapy; PARP1, poly(ADP-ribose) polymerase; TIC, tumour initiating cell.



down-regulation of DSB repair has been implicated as an underlying mechanism to this chromosomal fragility.<sup>61</sup> The compromise in DSB resolution in hypoxic cells and the resulting increase in chromosome aberrations is a “perfect storm” towards genomic instability in tumour cells that adapt and continue to proliferate under low oxygen conditions.<sup>27</sup> Hypoxia can also lead to a decrease in function of other DNA repair pathways, including that of mismatch repair (MMR), nucleotide excision repair and the Fanconi anaemia (FA) pathway.<sup>62–65</sup> The parallel reduction in various DNA damage repair pathways can all potentially contribute to the acquisition of aggressive tumour phenotypes.<sup>27</sup>

The genetic instability in hypoxic cells would at first seem a complex phenotype to target with standard or molecularly targeted therapies. Any clinical approach would require careful assessment of the tumour microenvironment and genomic status (using assays mentioned in previous sections) to incorporate both hypoxia and genomic assessment as part of a standard of care. However, the repair-defective phenotype might just be the undoing of the hypoxic resistance phenotype, providing an opportunity to specifically target hypoxic tumour cells and improve the therapeutic index.<sup>27</sup> Hypoxic tumour cells can be directly targeted using cytotoxic agents that induce DNA damage only under low oxygen; these include the bioreductive drugs, tirapazamine and

apaziquone.<sup>66,67</sup> A newer drug, TH-302 (a 2-nitroimidazole triggered hypoxia-activated cytotoxin) directly decreases the hypoxic fraction in xenografts of varying histology and is undergoing Phase II–III clinical trials in combination with chemotherapy.<sup>68</sup> Additional targeting of hypoxic subregions can be achieved by targeting HIF-1-dependent transcription or targeting the unfolded protein response, which controls gene translation under cellular stress (e.g. targeting the mechanistic target of rapamycin (mTOR) signalling pathway).<sup>69–71</sup>

One could also target the faulty DNA repair and genetic instability in hypoxic cells using the concept of “contextual” synthetic lethality.<sup>72</sup> “Genetic” synthetic lethality is a concept first developed using yeast genetics in which mutations in two genes (e.g. gene A and gene B) result in cell death, while a mutation in only one gene (e.g. either gene A or gene B) results in cell viability.<sup>73</sup> This concept has been successfully used to target tumours deficient in HR (e.g. *BRCA1*- or *BRCA2*-deficient ovarian cancer, breast cancer and CaPs) by the additional inhibition of the poly (ADP-ribose) polymerase (PARP1) protein.<sup>74</sup> PARP1 normally functions in single-strand break and base-excision repair, and its inhibition is synthetically lethal when the HR pathway is also compromised. Similarly, PARP1 inhibition can be toxic to the HR defects associated with hypoxic cells; hence the concept of “contextual” synthetic

lethality.<sup>72,75</sup> We have shown in principle that PARP1 inhibition can preferentially kill repair-defective hypoxic tumour clonogens, while sparing normal tissues that maintain their DNA repair capability. Other contextual synthetic lethal approaches could be the inhibition of DNA polymerase- $\beta$  in MMR-deficient hypoxic cells or inhibition of the ataxia telangiectasia mutated (ATM) kinase in hypoxic cells that have defective FA pathway function.<sup>8,75,76</sup>

Of interest, clinicians may already be combatting genetic instability and hypoxia in CaP with the combined use of ADT and RT.<sup>8</sup> This combination has led to improved overall and CaP-specific survival in high-risk and locally advanced CaP.<sup>8</sup> The use of neoadjuvant ADT (150 mg per day of bicalutamide) was shown to improve CaP oxygenation (based on pO<sub>2</sub> measurements) prior to RT.<sup>77</sup> Furthermore, three recent studies have suggested that ADT treatment reduces expression and function of the NHEJ and other DNA repair pathways, supporting the use of DNA-PKcs inhibitors in combination with ADT as a novel treatment for CaP.<sup>78–80</sup> Any approach that tries to increase cell kill in hypoxic cells using contextual synthetic lethality or alterations in DNA repair will require assays that can measure the function of NHEJ, HR, MMR and FA proteins *in situ* to ascertain the fraction of hypoxic cells within a tumour that may be repair deficient. These uses of the predictive biomarkers in addition to pharmacodynamic biomarkers that confirm drug activity *in vivo* will be required for maximal impact of the use of this targeted approach in combination with RT, surgery or chemotherapy.<sup>81</sup>

### A CAVEAT: THE PROBLEM OF PROSTATE CANCER MULTIFOCALITY AND STEM CELL SUBPOPULATIONS

CaP is unique in that it is a multifocal cancer with clonal subpopulations that have varied histological and molecular abnormalities that could relate to differential outcome. Heterogeneity exists both within and between patients. The vast majority of prostatectomies have more than one malignant focus within a prostate gland, which can be subcategorized by differential genomics based on *PTEN*, *c-MYC* and *NKX3.1* gene abnormalities, therefore containing CaP foci with differential prognostic information.<sup>34,82</sup> It is now well appreciated that tumours with identical GSs may exhibit profound genetic heterogeneity within a single prostate gland.<sup>44</sup> It is also unclear whether a focus that is being assayed will potentially fail treatment owing to local radioresistance or because it initially harboured a lethal metastatic clone. Anatomically distinct tumour metastases can be derived from a single progenitor clone;<sup>83–85</sup> a concept elegantly proven in renal cancer, whereby single needle biopsies did not predict the genetic heterogeneity within the primary tumour nor distant metastases for an individual patient.<sup>86</sup> Studies using circulating tumour cells and circulating cell-free DNA, RNA and miRNA, after improvements in assay specificity and sensitivity, could be useful as a means to detect the most aggressive features of CaP within so-called, “liquid biopsies”, as a function of staging, prognosis and treatment response.<sup>87–89</sup>

These findings must be also placed into the context of CaP tumour initiating cells (TICs), which constitute that subfraction of cells, which must be sterilized by RT to prevent tumour cell repopulation after treatment (*i.e.* treatment failure).<sup>90,91</sup> Pre-clinical studies suggest that CaP TICs may have increased biological growth under hypoxia and exist as a radioresistant hypoxic niche.<sup>92–94</sup> However, rigorous studies are required to delineate the exact TIC markers that will differentiate this subpopulation for specific genomic studies as this relates to individualized prognosis.<sup>95,96</sup> Finally, subtumoural heterogeneity in cancer metabolism (*e.g.* both acute and chronic hypoxia co-exist within a tumour and lead to significant gradients of oxygen consumption) could also confound quantitation and summary statistics for assaying the fraction of tumour hypoxia from one patient to another.<sup>14</sup> Intraprostatic heterogeneity must therefore be adequately “sampled” across multiple foci and TIC subpopulations with genomic or hypoxia assays, such that the most aggressive and key features of tumour progression and/or prognosis are not missed.

### CONCLUSIONS

A robust understanding of the interplay between hypoxia and genomics in the context of tumour heterogeneity is required to facilitate precision medicine for CaP. We must embrace these complexities if we are to target the most aggressive cases of CaP to improve cure rates (Figure 2). The genome-wide RNA- and DNA-based prognostic signatures developed and validated on post-treatment radical prostatectomy specimens must now be validated using pre-RT biopsies. The enormous reduction in cost and materials required for whole-genome and whole-transcriptome sequencing will further detail the genomics of CaP. Hypoxia-based assays must include functional assessment of DNA repair pathways if the concept of contextual synthetic lethality is to be acted upon within the clinic. Arranging the marriage between these biological end points will highlight key features of aggressive CaP variants that will best explain heterogeneous clinical outcome and provide novel treatments to offset both local and systemic resistance.

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### CONFLICTS OF INTEREST

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